

CLAIMS

We claim:

1. A method for determining the differences between the molecular interactions of two different mixtures of molecules, comprising:
  - labeling a first mixture of molecules and a target mixture of molecules;
  - introducing said first mixture of molecules to a combinatorial library of solid phase supports;
  - incubating said combinatorial library with said first mixture of molecules;
  - performing a first marking step to mark those of said solid phase supports that have a molecule of said first mixture bound to them;
  - introducing said target mixture of molecules to said combinatorial library;
  - incubating said combinatorial library with said target mixture of molecules;
  - obtaining a first image showing as marked those of said solid phase supports that have a molecule of said first mixture bound to them;
  - performing a second marking step to mark those of said solid phase supports that have a molecule of said target mixture bound to them;
  - obtaining a second image showing as marked those of said solid phase supports that have a molecule of said target mixture bound to them;

and

5 creating a third image identifying those of said solid phase supports that have  
a molecule of said target mixture bound to them, wherein said  
third image is created by comparing said first image and said  
second image.

10 2. The method of Claim 1, further comprising:

isолating one of said solid phase supports identified in said third image; and  
determining the chemical structure of a ligand on one of said isolated solid  
phase supports.

15 3. The method of Claim 1, wherein said first mixture of molecules is a protein extract  
from normal cells and said target mixture of molecules is a protein extract from  
cancer cells.

4. The method of Claim 1, wherein said combinatorial library is a one-bead-one-  
compound peptide library.

5. The method of Claim 1, wherein said labeling is performed by biotinylation.

6. The method of Claim 5, further comprising:

20 before said first marking step is performed, incubating said combinatorial  
library with a solution of streptavidin-alkaline phosphatase conjugate;  
and,

after said incubating said combinatorial library with said target mixture of  
molecules is performed, but before said obtaining said first image is

5 performed, incubating said combinatorial library with a solution of streptavidin-alkaline phosphatase conjugate.

7. The method of Claim 1, wherein said labeling is performed by the use of an antigen and corresponding antibody.
8. The method of Claim 1, wherein said first and said second marking steps are performed by incubating said combinatorial library in a solution of 5-bromo-4-chloro-3-indolyl-phosphate.
- 10 9. The method of Claim 1, wherein said combinatorial library is immobilized in a support matrix before said first image is obtained.
- 15 10. The method of Claim 1, wherein said first and said second images are graphical images, and said third image is created by comparing said first and said second images on a pixel-by-pixel basis.
11. The method of Claim 10, wherein said first image is image "A," said second image is image "B," and said third image is created by applying the formula  $(B-A)/A$  on a pixel-by-pixel basis.
- 20 12. A method for determining the differences between the molecular interactions of two different mixtures of molecules and identifying a ligand specific for a molecule in one of the mixtures, comprising:
- labeling a first mixture of molecules and a target mixture of molecules;

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introducing said first mixture of molecules to a combinatorial library of solid  
phase supports;

incubating said combinatorial library with said first mixture of molecules;  
performing a first marking step to mark those of said solid phase supports  
that have a molecule of said first mixture bound to them;

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introducing said target mixture of molecules to said combinatorial library;  
incubating said combinatorial library with said target mixture of molecules;  
obtaining a first image showing as marked those of said solid phase supports  
that have a molecule of said first mixture bound to them;

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performing a second marking step to mark those of said solid phase supports  
that have a molecule of said target mixture bound to them;  
obtaining a second image showing as marked those of said solid phase  
supports that have a molecule of said target mixture bound to them;

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creating a third image identifying those of said solid phase supports that have  
a molecule of said target mixture bound to them, wherein said  
third image is created by comparing said first image and said  
second image;

isolating one of said solid phase supports identified in said third image; and  
determining the chemical structure of a ligand on one of said isolated solid  
phase supports.

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13. The method of Claim 12, wherein said first and said second images are graphical images, and said third image is created by comparing said first and said second images on a pixel-by-pixel basis.
- 10 14. The method of Claim 13, wherein said first image is image "A," said second image is image "B," and said third image is created by applying the formula  $(B-A)/A$  on a pixel-by-pixel basis.
15. A method for identifying a ligand specific for a molecule in one of two different mixtures of molecules, comprising:
- labeling a first mixture of molecules and a target mixture of molecules;
- introducing said first mixture of molecules to a combinatorial library of solid phase supports;
- incubating said combinatorial library with said first mixture of molecules;
- performing a first marking step to mark those of said solid phase supports that have a molecule of said first mixture bound to them;
- introducing said target mixture of molecules to said combinatorial library;
- incubating said combinatorial library with said target mixture of molecules;
- obtaining a first image showing as marked those of said solid phase supports that have a molecule of said first mixture bound to them;
- performing a second marking step to mark those of said solid phase supports that have a molecule of said target mixture bound to them;

5                   obtaining a second image showing as marked those of said solid phase  
                      supports that have a molecule of said target mixture bound to them;  
                      creating a third image identifying those of said solid phase supports that have  
                      a molecule of said target mixture bound to them, wherein said  
                      third image is created by comparing said first image and said  
10                 second image;  
  
                     isolating one of said solid phase supports identified in said third image; and  
                      determining the chemical structure of a ligand on one of said isolated solid  
                      phase supports.

15. 16. A method for identifying a ligand specific for a target molecule, comprising:  
                     labeling a target molecule;  
                     incubating a combinatorial library of solid phase supports with a label  
                     binder;  
                     performing a first marking step to mark those of said solid phase supports  
                     that have a molecule of said label binder bound to them;  
                     introducing said target molecule to said combinatorial library;  
                     incubating said combinatorial library with said target molecule;  
                     obtaining a first image showing as marked those of said solid phase supports  
                     that were marked in said first marking step;

performing a second marking step to mark those of said solid phase supports

that have a target molecule bound to them;

obtaining a second image showing as marked those of said solid phase

supports that have a target molecule bound to them;

creating a third image identifying those of said solid phase supports that have

10 a target molecule bound to them, wherein said third image is created

by comparing said first image and said second image;

isolating one of said solid phase supports identified in said third image; and

determining the chemical structure of a ligand on one of said isolated solid

phase supports.

17. The method of Claim 16, wherein said labeling is performed by biotinylation, and further, wherein said label binder is streptavidin-alkaline phosphatase conjugate.

18. The method of Claim 16, wherein said labeling is performed by the use of an antigen and corresponding antibody.

19. A method of screening a combinatorial library, comprising:

labeling a target molecule;

incubating a combinatorial library of solid phase supports with a label

binder;

performing a first marking step to mark those of said solid phase supports

that have a molecule of said label binder bound to them;

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introducing said target molecule to said combinatorial library;  
incubating said combinatorial library with said target molecule;  
obtaining a first image showing as marked those of said solid phase supports  
that were marked in said first marking step;  
performing a second marking step to mark those of said solid phase supports  
10 that have a target molecule bound to them;  
obtaining a second image showing as marked those of said solid phase  
supports that have a target molecule bound to them; and  
creating a third image identifying those of said solid phase supports that have  
15 a target molecule bound to them, wherein said third image is created  
by comparing said first image and said second image.

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20. The method of Claim 19, further comprising:

isolating one of said solid phase supports identified in said third image; and  
determining the chemical structure of a ligand on one of said isolated solid  
phase supports.